LIPOSOME IMMUNOANALYSIS BY FLOW INJECTION ASSAY

This application is a continuation of application Ser. No. 07/473,020, filed Jan. 31, 1990, (abandoned) which in turn is a continuation-in-part of application Ser. No. 200,210, filed May 31, 1988, (abandoned).

BACKGROUND OF THE INVENTION

Antigen-antibody interactions are the basis of sensitive diagnostic techniques such as radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). Although these techniques are very sensitive and widely used, they are time-consuming and technically cumbersome. Commercial antibody-based diagnostic kits for hormone and drug detection are easier to use; some of these are disposable single-use devices that are semi-quantitative and permit direct observation of color changes. Such devices cannot be calibrated, however, which limits their analytical potential. A research approach currently being studied by a group headed by George Wilson at the University of Kansas employs immobilized antibodies in liquid chromatographic systems. In short, there are a very large number of configurations possible for immuno-based devices but for credible analytical applications, the optimal immuno-based assay system should be fast, reliable, sensitive, quantitative, reusable, and automated.

One approach to developing such a system involves the use of liposomes in a flow injection immunoassay.

Flow injection immunoanalysis (FIIA) is an analytical approach to immunoassays which combines immunochemistry with the technique of flow injection analysis (FIA). FIA is a continuous-flow method based on the introduction of a sample aliquot into a moving non-segmented carrier stream. Introductions and directions of flow may be determined by a series of microprocessor-controlled solenoid valves.

Immunoassays are of great importance because of their specificity toward analytes present in complex mixtures, and their high sensitivity. Most immunoassays involve the use of a fluorescent or chemiluminescent, enzyme, or radioactive label on an immunoreactive species which serves as an indicator that an immunospecific reaction has occurred. Such immunoassays are discussed in U.S. Pat. Nos. 4,372,745; 4,551,426; and 4,108,976. Immunoassays can be divided into two broad categories, heterogeneous (solid phase) assays and homogeneous (solution phase) assays. Heterogeneous immunoassays have been developed which use the three types of labels previously mentioned. Certain fluorescent and enzyme tags are also used in homogeneous immunoassays, but these uses are limited to small ligands.

Enzyme-linked immunosorbant assays (ELISAs) involve the use of an enzyme covalently coupled to an immunoreactive reagent to serve as an indicator that an immunospecific reaction has occurred. The enzyme is linked to a secondary reagent, which is added to the assay after the initial immunochemical interaction between the analyte or ligand and the antibody. The sensitivity of ELISA is due to the number of turnover events the enzyme is capable of during an incubation period with a substrate that is cleaved to a colored product. While ELISA can be extremely sensitive, it is frequently a very time-consuming assay.

Uses for liposomes in immunoassays have been developed. Liposomes are spherical membrane structures which form spontaneously when phospholipid molecules are dispersed in water. The bilayer membranes of liposomes are similar to cellular membranes and surround an entrapped aqueous volume. Markers, such as water-soluble fluorescent molecules, such as the dye carboxyfluorescein, or electroactive markers, such as potassium ferrocyanide, are trapped inside the lipid membrane, and non-trapped material is removed from the outside of the structures by gel filtration. There are a number of methods established for liposome preparation. Liposomes prepared having a bilayer thickness of 40 angstroms, at higher concentrations, may contain approximately 1×10^5 carboxyfluorescein molecules within.

The detection of binding of antigen analytes to antibodies may be accomplished by the use of radioactive or fluorophore- or enzyme- tagged molecules. Modification usually involves one to several fluorophores per molecule or one enzyme which is capable of several hundred turnover events in a reasonable period of time. Radioactivity is more sensitive, since many decay events can be counted, but there are many disadvantages to radio labels. Labeling analyte analogs or ligand binding proteins such as antibodies with liposomes containing fluoroescent or electro-active molecules is analogous to labeling these agents with radioisotopes or enzymes. The advantage of liposomes is that they provide real-time detectability of up to 1×10^5 entrapped molecules for every molecule event they are associated with. The use of liposomes provides as much sensitivity as radio labels but is safer, faster and more convenient. Some preparations are extremely stable, with no aggregating and no detectable change in size or other characteristics after storage for years.

Some assays take place in solution, and involve immunospecific lysis of liposomes in the presence of interacting immunochemical species, one of which is the desired analyte, and a complex biologically derived lytic reagent called complement. These assays are discussed in U.S. Pat. Nos. 4,707,441 and 4,483,929. A solid-phase liposome immunoassay which involves immunospecific disruption of liposomes is discussed in U.S. Pat. No. 4,708,933.

U.S. Pat. No. 4,708,933 discloses an immunoliposome assay wherein liposomes are modified to contain antigen and dye. When these liposomes come into contact with an inert solid surface having antibody molecules attached thereto, rapid binding occurs between the antigen-lipid complex and the antibody, disrupting the liposome and releasing the dye which can be quantitatively measured.

Liposomes can also be used in homogeneous assays. These assays involve incorporating fluorophores or enzymes into liposomes, and modulating the detectable signal as a result of an immunospecific response. In this case, the immunospecific response requires the presence of interacting immunochemical species, one of which is the desired analyte, and a complex biologically derived lytic reagent called complement. Such assays are discussed in U.S. Pat. Nos. 4,707,441 and 4,483,929. Some immunoassays are based on the immunospecific aggregation of latex particles. Latex particles must be covalently derivatized with immunoreactive reagents, and the stability of latex particles after long-term storage is a problem. A need arises for an immunoassay which overcomes these problems.